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Emergence of Trimethoprim Resistance in Fecal Flora

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The emergence of trimethoprim (TMP) resistance in fecal flora was compared in patients with urinary tract infection treated with TMP or TMP-sulfamethoxazole. No significant differences were found in the occurrence of TMP-resistant fecal aerobic bacteria in the two treatment groups before and after treatment.

The effect of trimethoprim (TMP) or TMP-sulfamethoxazole (SMX) treatment on the emergence of fecal bacteria has been studied by many investigators in different countries (2, 4, 8, 10, 14, 15, 17–19). With one exception, little or no emergence of fecal TMP-resistant strains has been found in these studies. Different findings were presented by Murray et al., who compared TMP with TMP-SMX and placebo in the prophylaxis of diarrhea among students travelling to Mexico (14). After prophylaxis with TMP or TMP-SMX, the number of TMP-resistant *Escherichia coli* organisms increased markedly in both groups; in the placebo group, no increase was seen. The purpose of the present study was to evaluate the effect on fecal flora in outpatients in Finland of a 10-day treatment of urinary tract infection (UTI) with TMP or TMP-SMX.

Ninety-seven patients were studied between June and December 1982 at the Posio and Ranua Health Centers in Finland. All patients aged 16 to 64 years with acute, uncomplicated UTI were included. No antimicrobial treatment within the preceding month or treatment with TMP or SMX within the previous 3 months was allowed. Patients with known structural abnormalities of the urinary tract were excluded. Patients were alternately assigned to the TMP or TMP-SMX group. Only patients allergic to SMX changed the assignment order. TMP was used at a dose of 160 mg twice a day, and TMP-SMX was used at a dose of 160 mg + 800 mg, respectively, twice a day (Trimopan and Trimosulfa; Lääkefarmos Ltd., Turku, Finland). Both treatments were continued for 10 days. Treatment of two patients was interrupted because of skin reactions (one in the TMP group and one in the TMP-SMX group).

Routine quantitative urine cultures were carried out (Uricult-dipslide procedure; Orion Diagnostica, Espoo, Finland). Stool samples were obtained before therapy, at the end of therapy (±1 day), and 1 month (25 to 32 days) after the end of therapy. Stool (0.5 g) was weighed, serially diluted in physiological saline, and then plated on CLED agar (Oxoid, Basingstone, Hampshire, England), MacConkey agar (Difco Laboratories, Detroit, Mich.), and lactose agar (base agar with 15 g of lactose per 1,000 ml and 1.6% bromcresol purple). All colonies differing in appearance were studied further. Bacterial strains were identified by normal routine methods including Analytical Profile Index procedures (Analytab Products, Plainview, N.Y.; 11). A total of 956 different isolates were tested further (an average of 3.4 different strains in the first sample, 4.1 in the second sample, and 4.2 in the third sample). Susceptibility tests were carried out by the agar plate dilution method using PDM-ASM agar (AB Biodisk, Solna, Sweden) with doubling concentrations of TMP-lactate (0.125 to 1,024 μg/ml; Burroughs Wellcome Co., Research Triangle Park, N.C.) and SMX (2 to 1,024 μg/ml; Lääkefarmos [3]). Standard inocula of 5×10^4 to 5×10^5 CFU/ml were controlled by the most-probable-number method (9). After multipoint inoculation, plates were incubated at 35°C for 18 h (6). Resistance breakpoints used were 8 μg/ml for TMP and 512 μg/ml for SMX (6). *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains. The occurrence of homological DNA sequences to TMP resistance transposon Tn*7* was studied by DNA-DNA colony hybridization with a *Bam*HI fragment of ColE1::Tn*7* as a DNA probe (16). Positive colony hybridization together with a MIC of >1,024 μg of TMP per ml strongly suggested transposon Tn*7*-mediated resistance.

UTIs were caused by *E. coli* (61%), *Staphylococcus saprophyticus* (13%), *Proteus mirabilis* (3%), *Enterobacter* spp. (2%), and *Staphylococcus epidermidis* (1%). In 13% of patients, urine cultures considered negative (<10^5 CFU/ml of urine) were obtained, and in 6% of patients, cultures

TABLE 1. Appearance of TMP resistance in fecal samples

Group and time of sample	No. of patients	% of patients with TMP-resistant strains of:		
		Total	Enterobacteria	Other isolates
TMP group				
Before treatment	49	14.3	12.2	2.0
Immediately after treatment	43	46.5	27.9	20.9
1 month after treatment	34	14.7	11.8	2.9
TMP-SMX group				
Before treatment	44	11.6[a]	6.8[a]	6.8[a]
Immediately after treatment	44	40.9[a]	22.7[a]	27.3[a]
1 month after treatment	35	11.4[a]	2.9[a]	8.6[a]

[a] Difference was not significant ($P > 0.05$) when compared with the corresponding samples in the TMP group (binomic *t*-test).

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TABLE 2. Appearance of SMX resistance in fecal samples

Group and time of sample	No. of patients	% of patients with SMX-resistant strains of:		
		Total	Enterobacteria	Other isolates
TMP group				
Before treatment	49	38.8	14.3	30.6
Immediately after treatment	43	48.8	7.0	44.2
1 month after treatment	34	55.9	17.6	44.1
TMP-SMX group				
Before treatment	44	38.6[a]	9.1[a]	34.1[a]
Immediately after treatment	44	77.3[b]	11.4[a]	70.5[c]
1 month after treatment	35	60.0[a]	17.1[a]	57.1[a]

[a] Difference was not significant ($P > 0.05$) when compared with the corresponding samples in the TMP group.
[b] $P < 0.01$.
[c] $P < 0.05$.

considered as mixed flora (more than three different species) were obtained. All causative bacterial strains were susceptible to TMP and SMX. Only one case of therapeutic failure was observed in each group; in the TMP group, reinfection was caused by TMP-susceptible *S. saprophyticus*, and in the TMP-SMX group, reinfection was caused by TMP- and SMX-susceptible *E. coli*.

Reduction of the aerobic fecal flora was clear in both treatment groups (an average reduction of from 6 log to 2 or 3 log colony counts per g of stool). With TMP-resistant bacteria, no significant difference between TMP and TMP-SMX groups was observed (Table 1). Only immediately after the treatment was a significant difference in the occurrence of SMX-resistant strains observed; SMX-resistant strains occurred in 48.8 and 77.3% of patients in the TMP and TMP-SMX groups, respectively ($P < 0.01$; Table 2), and the increase was due mainly to the increase of enterococci.

All TMP-resistant enterobacteria ($n = 26$) with MICs of 8 μg or more of TMP per ml were studied further by DNA hybridization. Only three strains showed homology with the Tn7 probe. These three isolates were highly resistant to TMP (MIC, >1,024 μg/ml) and originated in the TMP-SMX group. Two of these strains were also SMX resistant. Only 3 of the DNA hybridization-negative strains had MICs of > 1,024 μg/ml, and the MICs of the remaining 20 strains varied evenly between 8 and 512 μg/ml. Of the 26 TMP-resistant strains, 8 were SMX resistant.

It is of interest that in developed countries, like England and Finland, TMP resistance does not appear to have increased to the same extent it has in the developing countries. TMP resistance in samples of urinary tract *E. coli* collected in Finland from patients with UTI is still between 4.0 and 10.1% (P. Huovinen, M.D. thesis, University of Turku, Turku, Finland, 1984). In England, the corresponding figures for gram-negative urinary pathogens were 5.4 and 11% (1, 13). It remains unclear why results of the present study are different from the results from Mexico of Murray et al. (14). The most probable reason is the wide use, without prescription, of antimicrobial agents in Mexico and the concomitant occurrence of multiply resistant microorganisms. Although the total amount of TMP consumed annually in outpatients in Finland (with a total population of 4.8 million) was nearly 2,000 kg in 1983 and the total amount consumed in Mexico (with a total population of 73 million) was officially about 6,100 kg in 1980, the emergence of TMP-resistant enterobacteria in fecal samples during therapy with TMP or TMP-SMX is strikingly different (12). However, the amount of TMP and TMP-SMX sold with no controls or for animals in Mexico is unknown.

Our results reveal an increase of TMP-resistant enterobacteria in Finland since 1976, when only one TMP-resistant coliform was found in the TMP group after 4 weeks of treatment (18). However, in both treatment groups in this study, SMX resistance seems to be at the same level as in 1976.

To evaluate the usefulness of TMP in the treatment of UTIs, a continuous follow-up is needed. We do not recommend the use of TMP in hospitals or areas where the prevalence of TMP resistance is high (5–7). However, at present, TMP and TMP-SMX seem to be useful antimicrobial agents in the treatment of UTIs in Finland, as clinically significant emergence of TMP resistance during therapy of outpatients thus far has not been documented.

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